REMARKS

I. Status Summary

Claims 12 and 24-32 are pending in the subject application. Claims 12 and 24-32 currently stand rejected.

Claims 12 and 24-29 have been rejected under 35 U.S.C. §102(a) as allegedly being anticipated by the publication Jonuleit *et al.* (2000) *J. Exp. Med.* **192**(9): 1213-1222 (hereinafter referred to as "Jonuleit *et al.*").

Claims 12 and 24-30 have been rejected under 35 U.S.C. §102(e) as allegedly being anticipated by U.S. Patent No. 6,803,036 to Horwitz *et al.* (hereinafter referred to as "Horwitz *et al.*").

Claim 31 has been rejected under 35 U.S.C. §103(a) as allegedly being obvious over Horwitz *et al.*

Claim 32 has been rejected under 35 U.S.C. §103(a) as allegedly being obvious over Horwitz *et al.* in view of Jonuleit *et al.*

Claim 12 has been amended herein. Support for the amendment to claim 12 can be found throughout the specification as filed, including particularly at page 3, line 24; and page 7, lines 5-6. No new matter has been added.

II. Response to the 35 U.S.C. §102(a) Rejection of Claims 12 and 24-29 Based Upon Jonuleit *et al.*

Claims 12 and 24-29 have been rejected under 35 U.S.C. §102(a) as allegedly being anticipated by Jonuleit *et al.* Particularly, the Patent Office asserts that on page 1214 (second column, 4th paragraph, lines 1-6, and Figure 4), Jonuleit *et al.* discloses a method of identifying, monitoring, and/or removing $CD4^+$ $CD25^+$ cells from human blood by contacting the blood with CD4 and/or CD25 and/or CTLA-4 specific antibodies.

After careful review of the instant rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Preliminarily, applicants note that it is well settled that for a cited reference to qualify as prior art under 35 U.S.C. §102, each element of the claimed subject matter must be disclosed within the reference. "It is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention." Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986).

Further, Jonuleit et al. do not teach the removal of $CD4^+CD25^+$ regulatory T cells from human blood by contacting the blood with CD4 and CD25 and/or CTLA-4 specific antibodies, as presently claimed. Rather, as described in the passage cited in the Office Action (page 1214, second column, 4th paragraph, lines 1-6), Jonuleit et al. teach the removal of naïve $CD4^+$ T cells from cord blood using CD4 MACS beads alone and the removal of naïve $CD4^+$ T cells from peripheral blood using a CD4/CD45RA Multisort kit. CD45RA is not the equivalent of CD25 or CTLA-4. Figure 4, which is also cited in the Office Action, shows that alloreactive T cells have a $CD25^+$ phenotype, but this phenotype is exhibited after stimulation of the T cells in vitro by coculturing them with allogeneic DCs for 42 hours (see Figure 4 legend, page 1216). Accordingly, Jonuleit does not teach the isolation of $CD4^+$ $CD25^+$ T regulatory cells from human blood.

Applicants also note that claim 12 (and thus also claims 24-29, which depend from or incorporate the elements of claim 12) has been amended in response to the comments in the last Office Action to clarify, as previously discussed, that the claimed method is directed to the isolation of $CD4^+$ $CD25^+$ regulatory T cells that are present in human blood. Support for this amendment can be found throughout the specification as well as specifically, for example, on page 3, line 24, and on page 7, lines 5-6. No new matter has been added.

Applicants respectfully submit that the amendment to independent claim 12 distinguishes the presently claimed subject matter over Jonuleit *et al.* Particularly, independent claim 12 recites a method of identifying, monitoring and/or removing $CD4^+$ $CD25^+$ regulatory T cells present in human blood. In marked contrast, Jonuleit *et al.* is believed to teach the induction of regulatory T cells from human naïve T cells through repetitive stimulation with immature dendritic cells. Thus, applicants

respectfully submit that in contrast to the regulatory T cells disclosed in the method of independent claim 12, the cell population disclosed in Jonuleit *et al.* is non-naturally occurring.

Applicants further submit that Jonuleit *et al.* discloses additional details that indicate further differences between the naturally occurring $CD4^+$ $CD25^+$ regulatory T cells of the present claims and the cells produced by Jonuleit *et al.* Particularly, applicants respectfully submit that the cells isolated from blood in Jonuleit *et al.* are not $CD4^+$ $CD25^+$ regulatory T cells. Rather, Jonuleit *et al.* is believed to teach that naïve $CD4^+$ T cells are first either purified from cord blood using CD4 MACS beads, or positively selected from peripheral blood using a CD4/CD45RA Multisort kit. See, for example, page 1214, column 2. Jonuleit *et al.* thus teaches the following procedure for the *in vitro* generation of cells with a cytokine profile characteristic of Tr1 cells and with regulatory function at page 1216, column 2, through page 1217, column 1:

> [N]aive T cells primed and restimulated with allogeneic iDC showed a Th0 cytokine profile after the first restimulation (synthesis of intermediate amounts of IFNγ and IL-4). After repetitive stimulation with iDCs, the alloreactive T cells lost their capacity to synthesize IFN-γ, IL-2 and IL-4, indicating that these T cells did not differentiate into Th2 cells, but showed, however, an enhanced production of IL-10. Thus, the respective T cell progeny exhibited a cytokine profile characteristic of Tr1 cells, i.e., synthesis of high amounts of IL-10 and no or negligible production of IFN-γ, IL-2, IL-4 or IL-5. * * *
>
> As shown in Fig. 6, Tr1-like cells suppressed the proliferation of syngeneic Th1 cells in response to allogeneic mDCs in a dose dependent manner.

Thus, applicants respectfully submit that Jonuleit *et al.* at best teach the *in vitro* generation of Tr1-like cells through repetitive stimulation with allogeneic iDCs. Applicants further submit that the title of Jonuleit *et al.* ("Induction of Interleukin 10-producing, Nonproliferating $CD4^+$ T Cells with Regulatory Properties by Repetitive Stimulation with Allogeneic Immature Human Dendritic Cells") further emphasizes

that Jonuleit *et al.* teaches the production of cells having the above-specified properties, rather than the isolation of a natural population.

Accordingly, applicants respectfully submit that Jonuleit *et al.* at best teaches an *in vitro* method of producing a particular cell population and does not teach the isolation of a natural population of human $CD4^+$ $CD25^+$ regulatory T cells, as presently set forth in independent claim 12. In view of the above-cited differences between Jonuleit *et al.* and the presently claimed subject matter, applicants respectfully submit that Jonuleit *et al.* does not teach each and every element of independent claim 12. Accordingly, applicants respectfully submit that the instant 35 U.S.C. §102(a) rejection of independent claim 12 has been addressed. Applicants further respectfully request that the instant rejection of independent claim 12 be withdrawn at this time, and a Notice of Allowance issued.

In view of the dependency of claims 24-32 from independent claim 12, applicants respectfully submit that the 35 U.S.C. §102(a) rejection of claims 24-32 over Jonuleit *et al.* has also been addressed. Applicants further respectfully request that the instant rejection of claims 24-32 be withdrawn at this time and a Notice of Allowance issued.

III. Response to the 35 U.S.C. §102(e) Rejection of Claims 12 and 24-30 Based On Horwitz *et al.*

The Patent Office has rejected claims 12 and 24-30 under 35 U.S.C. §102(e) as allegedly being anticipated by Horwitz *et al.* Particularly, the Patent Office asserts that Horwitz *et al* discloses a method of identifying, monitoring, and/or removing $CD4^+$ $CD25^+$ cells from human blood by contacting the blood with CD4 and/or CD25 and/or CTL-A4 specific antibodies.

After careful review of the instant rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Preliminarily, as set forth hereinabove, applicants respectfully submit that for the cited reference to be an anticipation reference under 35 U.S.C. §102, the reference must disclose each and every element of the claimed subject matter.

Contrary to the Patent Office's assertions, applicants respectfully submit that Horwitz *et al.* does not disclose or teach the removal of $CD4^+$ $CD25^+$ regulatory T cells from human blood by contacting the blood with CD4 and CD25 and/or CTLA-4 specific antibodies, as set forth in independent claim 12. Rather, Horwitz *et al.* teaches the *in vitro* induction of regulatory T cells. Specifically, Horwitz *et al.* teach the removal of naïve $CD4^+$ T cells from blood, after which the cells are "incubated [*i.e.*, cultured *in vitro*] with [a] suppressive-inducing composition..." See, for example, column 11, lines 5-6 and column 13, lines 11-14. Horwitz *et al.* further teaches that "[i]n a preferred embodiment, the invention provides methods for treating T cells with a suppressive-inducing composition and T cell activators, to generate regulatory T cells." See, for example, column 14, lines 9-11.

Applicants further respectfully submit that the Horwitz *et al.* passages cited by the Patent Office in support of the instant rejection describe the *in vitro* induction of regulatory T cells and their use in various experiments. Specifically, applicants respectfully submit that Figure 9 of Horwitz *et al.* illustrates the results from experiments using naïve $CD4^+$ T cells that were cultured for 5 days prior to the experiment and further demonstrates "that activation of naïve $CD4^+$ T cells in the presence of TGF-β enables them to respond more vigorously to alloantigens." See Figure 9 legend, in column 7, lines 49-65. Similarly, Figure 10A shows results from experiments using "[n]aive $CD4^+$ T cells primed with irradiated allogeneic stimulator cells ± TGF- β." After the *in vitro* treatment, the cells were sorted into $CD25^+$ and $CD25^-$ fractions (see Figure 10A legend in col. 7, line 66 through col. 8, line 3)[a]. Further, applicants respectfully submit that Figure 7 of Horwitz *et al.* demonstrates that only after induction by TGF- β were $CD4^+$ regulatory T cells shown to have suppressive activity. See, for example, column 21, lines 25-26. Thus, applicants

[a] The Figure 10A legend (col. 7, line 66 through col. 8, line 3) clarifies that the experiments discussed in col. 21, lines 60-67, cited in the Office Action, are the same experiments.

submit that the particular portions of Horwitz *et al.* cited by the Patent Office in support of the instant rejection at best describe experiments wherein naïve $CD4^+$ T cells were cultured *in vitro* to induce a regulatory phenotype.

As indicated hereinabove, independent claim 12 has been amended to recite a method to identify, monitor and/or remove CD4+ CD25+ regulatory T cells from human blood comprising the step of contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells, whereby CD4+ CD25+ regulatory T cells present in human blood are identified, monitored, and/or removed from the human blood. Support for the amendment to claim 12 can be found throughout the specification as filed, including particularly at page 3, line 24; and page 7, lines 5-6. No new matter has been added.

Applicants further point to additional passages in Horwitz *et al.* that are believed to emphasize that cells having regulatory properties can be produced from naïve $CD4^+$ T cells following *in vitro* culture. For example, at column 10, lines 52-56, Horwitz *et al.* recites:

> Using the methods of the present invention, tolerance is established by treating a population of T cells ex vivo with a suppressive-inducing composition. Regulatory cells are generated by treating a population of T cells ex vivo with an activating agent and a suppressive-inducing composition.

Horwitz *et al.* further describes the treatment of the isolated naïve T cells at column 13, lines 9-24:

> Once the cells have undergone any necessary pretreatment, the cells are treated with a suppressive-inducing composition. By "treated" in this context herein is meant that the cells are incubated with the suppressive-inducing composition for a time period sufficient to result in T cell tolerance, particularly when transplanted into the recipient patient. The incubation will generally be under physiological temperature.
>
> A suppressive-inducing composition includes at least one compound which induces T cells to become tolerant to a recipients cells. By "suppressive-inducing composition" herein is meant a composition that can induce T cell

> tolerance. Generally, these compositions are cytokines. Suitable suppressive-inducing compositions include, but are not limited to, IL-10, IL-2, IL4, IL15 and TGF-β.

Accordingly, applicants respectfully submit that Horwitz *et al.* does not disclose or teach the removal of $CD4^+$ $CD25^+$ regulatory T cells from human blood by contacting the blood with CD4 and CD25 and/or CTLA-4 specific antibodies, as recited in independent claim 12. Rather, Horwitz *et al.* at best teaches the *in vitro* induction of regulatory T cells from $CD4^+$ T cells through stimulation with TGF-β, which is believed to be a non-naturally occurring population. Accordingly, applicants respectfully submit that the instant 35 U.S.C. §102(e) rejection of independent claim 12 over Horwitz *et al.* has been addressed. Applicants further respectfully request that the instant rejection of independent claim 12 be withdrawn at this time and a Notice of Allowance issued.

In view of the dependency of claims 24-30 from independent claim 12, applicants respectfully submit that the 35 U.S.C. §102(e) rejection of claims 24-30 over Horwitz *et al.* has also been addressed. Applicants further respectfully request that the instant rejection of claims 24-30 be withdrawn at this time and a Notice of Allowance issued.

IV. Response to 35 U.S.C. §103 Rejections

IV.A. Response to the Rejection of Claim 31 Based On Horwitz *et al.*

The Patent Office has rejected claim 31 under 35 U.S.C. §103(a) as allegedly being unpatentable over Horwitz *et al.* Particularly, the Patent Office asserts that Horwitz *et al.* teaches that $CD4^+$ $CD25^+$ cells are activated. The Patent Office concedes that Horwitz *et al.* does not teach that the $CD4^+$ $CD25^+$ cells are activated and fixed. However, the Patent Office asserts that it would have been obvious to one of ordinary skill in the art to use a population of fixed and activated $CD4^+$ $CD25^+$ cells to test regulatory activity because such cells can be viewed under a microscope.

After careful review of the instant rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Initially, applicants submit that in order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation in the references themselves to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. Manual of Patent Examining Procedures (M.P.E.P.) 2142; *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

As discussed herein above, independent claim 12 has been amended herein to disclose a method to identify, monitor and/or remove CD4+ CD25+ regulatory T cells from human blood comprising the step of contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells, whereby CD4+ CD25+ regulatory T cells present in human blood are identified, monitored, and/or removed from the human blood. Support for the amendment to independent claim 12 can be found throughout the specification as filed, including particularly at page 3, line 24; and page 7, lines 5-6. No new matter has been added. Applicants further submit that claim 31 is dependent upon independent claim 12, and as such, the amendment to claim 12 can be applied to claim 31.

As also discussed above, Horwitz *et al.* is not believed to teach the presently claimed method of identifying, monitoring and/or removing $CD4^+$ $CD25^+$ regulatory T cells present in human blood. Rather, Horwitz *et al.* is believed to teach the *in vitro* induction of regulatory T cells from $CD4^+$ T cells through stimulation with TGF-β. Further, the population described in Horwitz *et al.* is believed to be a non-naturally occurring population. Accordingly, applicants respectfully submit that Horwitz *et al.* does not teach each and every element of the method disclosed in independent

claim 12. Therefore, applicants respectfully submit that in view of the dependency of claim 31 from independent claim 12, Horwitz *et al.* fails to teach the method set forth in claim 31, wherein the cells obtained from the method of claim 12 are further activated and fixed.

In view of the analysis set forth hereinabove, the 35 U.S.C. §103(a) rejection of claim 31 in view of Horwitz *et al.* is believed to have been addressed. Accordingly, applicants respectfully request that the instant rejection of claim 31 be withdrawn at this time and a Notice of Allowance issued.

IV.B. Response to the Rejection of Claim 32 Based on Horwitz *et al.* in view of Jonuleit *et al.*

The Patent Office has rejected claim 32 under 35 U.S.C. §103(a) as allegedly being unpatentable over Horwitz *et al.* in view of Jonuleit *et al.* Particularly, the Patent Office asserts that Horwitz *et al.* teaches each element of claim 32, except analyzing $CD4^+$ $CD25^+$ cells for a cytokine profile of predominant secretion of IL-10 and only low levels of IL-2, IL-4, and IFN-γ. However, the Patent Office asserts that Jonuleit *et al.* teaches that enhanced production of IL-10 and low production of IL-2, IL-4, and IFN-γ is characteristic of Tr1 cells. The Patent Office further asserts that it would have been obvious to one of ordinary skill in the art to test the cytokine profile of predominant secretion of IL-10 and only low levels of IL-2, IL-4, and IFN-γ of the $CF4^+$ $CD25^+$ cells taught by Horwitz *et al.* because it is a characteristic of Tr1 cells.

After careful review of the instant rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Initially, as discussed herein above, applicants submit that independent claim 12 has been amended to disclose a method to identify, monitor and/or remove CD4+ CD25+ regulatory T cells from human blood comprising the step of contacting the human blood with ligands specifically binding to the CD4 and CD25, and/or CTL-A4 entities on the T cells, whereby CD4+ CD25+ regulatory T cells present in human blood are identified, monitored, and/or removed from the human blood. Support for the amendment to independent claim 12 can be found throughout the specification as

filed, including particularly at page 3, line 24; and page 7, lines 5-6. No new matter has been added. Applicants submit that claim 32 is dependent upon independent claim 12, and as such, the amendment to claim 12 can be applied to claim 32.

As also discussed in detail hereinabove, neither Horwitz *et al.* nor Jonuleit *et al.* are believed to teach the presently claimed method of identifying, monitoring and/or removing $CD4^+$ $CD25^+$ regulatory T cells present in human blood. Rather, Horwitz *et al.* and Jonuleit *et al.*, either alone or in combination, are believed to at best teach the *in vitro* induction of regulatory T cells from $CD4^+$ T cells by various means. Further, the populations described by Horwitz *et al.* and/or Jonuleit *et al.* are non-naturally occurring populations. Accordingly, applicants respectfully submit that Horwitz *et al.* and Jonuleit *et al.*, either alone or in combination, do not teach each and every element of independent claim 12.

Further, in view of the dependency of claim 32 from independent claim 12, applicants further submit that Horwitz *et al.* and/or Jonuleit *et al.* do not teach each and every element of claim 32. Accordingly, applicants respectfully submit that the instant 35 U.S.C. §103(a) rejection of claim 32 over Horwitz *et al.* in view of Jonuleit *et al.* has been addressed. As such, applicants respectfully request that the instant rejection of claim 32 be withdrawn, and a Notice of Allowance issued at this time.

CONCLUSION

In light of the above amendments and remarks, it is respectfully submitted that the present application is now in proper condition for allowance, and an early notice to such effect is earnestly solicited.

If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

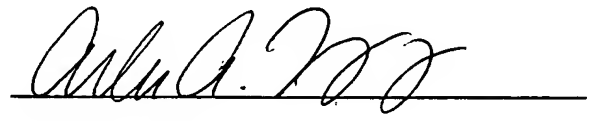
DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any fees associated with the filing of this correspondence to Deposit Account No. **50-0426**.

Respectfully submitted,

JENKINS, WILSON, TAYLOR & HUNT, P.A.

Date: 1/30/2007

By: 

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